09/825,857

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=> s probe#(10a)cleav####(10a)(tag# or reporter#)
L1          60 PROBE#(10A) CLEAV####(10A)(TAG# OR REPORTER#)

=> s l1 and ((produc### or generat###)(10a)(singlet or peroxide or NADH or
hydroxyl))
L2           1 L1 AND ((PRODUC### OR GENERAT###)(10A)(SINGLET OR PEROXIDE OR
               NADH OR HYDROXYL))

=> d l2 bib ab kwic

L2   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS
AN   2003:376266  CAPLUS
TI   Methods for detecting a plurality of analytes by chromatography
IN   Chenna, Ahmed; Matray, Tracy J.; Hernandez, Vincent S.; Hooper, Herbert;
     Singh, Sharat
PA   USA
SO   U.S. Pat. Appl. Publ., 26 pp.
     CODEN: USXXCO
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.         KIND  DATE           APPLICATION NO.  DATE
     ---------------    ----  --------       ---------------  --------
PI   US 2003092012      A1    20030515       US 2001-10949    20011109
     WO 2003042398      A2    20030522       WO 2002-US35864  20021108
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
             PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
             UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,
             TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
             CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
             PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
             NE, SN, TD, TG
PRAI US 2001-10949      A     20011109
AB   The invention provides a method for detecting a target nucleic acid
     sequence or other analyte such as protein, peptide, polysaccharide, lipid,
     or small mol.  The method involves contacting one or more target nucleic
     acid sequences with a set of tagged probes under conditions sufficient for
     hybridization of a target nucleic acid sequence with a tagged probe, the
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tagged probes comprising a mobility modifier attached to a nucleic acid target binding moiety by a bond that is cleavable by a nuclease, the nucleic acid target binding moiety contg. at least one bond resistant to said nuclease; treating the tagged probe hybridized to the target nucleic acid with a nuclease under conditions sufficient for cleavage of the nuclease-cleavable bond to release a tag reporter; sepg. a tag reporter using a chromatog. method, and detecting a tag reporter corresponding to a known target sequence. The tagged probe may also comprise a mobility modifier attached to a target binding moiety by a bond that is cleavable by visible light. A multiplexed sandwich immunoassay for six cytokines (IL-4, IL-6, IL-8, IL-10, TNF.alpha., and IFN.gamma.) was conducted using antibodies each tagged with a specific different light-cleavable carboxyfluorescein-derived tag (prepn. given) and second antibodies conjugated to a sensitizer. Released tags were sepd. using HPLC and detected using a fluorescence detector.

IT INDEXING IN PROGRESS

IT Immunoglobulins

RL: RCT (Reactant); RACT (Reactant or reagent)
(G, conjugation with **tags**; analytes detection by chromatog. using **probes cleavably** tagged with mobility modifiers)

IT 7782-44-7, Oxygen

RL: FMU (Formation, unclassified); RCT (Reactant); FORM (Formation, nonpreparative); RACT (Reactant or reagent)
(**singlet**, cleavable group contg. sensitizer **generating**; analytes detection by chromatog. using probes cleavably tagged with mobility modifiers)

=> s l2 and kit#
L3 0 L2 AND KIT#

=>

09/825,851

WEST

Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: L2 and kit$1

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

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Search History

DATE: Wednesday, May 28, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L3	L2 and kit$1	1	L3
L2	L1 and (generat$3 or produc$3) near5 (singlet or peroxide$1 or NADH or hydroxy)	1	L2
L1	probe$1 near5 cleav$4 near5 (tag$1 or reporter$1)	277	L1

END OF SEARCH HISTORY

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L3: Entry 1 of 1 File: USPT Feb 4, 2003

US-PAT-NO: 6514700
DOCUMENT-IDENTIFIER: US 6514700 B1

TITLE: Nucleic acid detection using degradation of a tagged sequence

DATE-ISSUED: February 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Singh; Sharat	San Jose	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
ACLARA BioSciences, Inc.	Mountain View	CA			02

APPL-NO: 09/ 602586 [PALM]
DATE FILED: June 21, 2000

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of application Ser. No. 09/561,579, filed Apr. 28, 2000 now abandoned, which is a continuation-in-part of application Ser. No. 09/303,029, filed Apr. 30, 1999, now U.S. Pat. No. 6,322,980 which disclosure is incorporated herein by reference.

INT-CL: [07] C12 Q 1/68

US-CL-ISSUED: 435/6
US-CL-CURRENT: 435/6

FIELD-OF-SEARCH: 435/6, 435/91.2, 536/22.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4274240	June 1981	Soum	
☐	4675300	June 1987	Zare et al.	
☐	5324401	June 1994	Yeung et al.	
☐	5470705	November 1995	Grossman et al.	
☐	5552028	September 1996	Madabhushi et al.	
☐	5560811	October 1996	Briggs et al.	
☐	5565324	October 1996	Still et al.	
☐	5567292	October 1996	Madabhushi et al.	
☐	5580732	December 1996	Grossman et al.	
☐	5624800	April 1997	Grossman et al.	
☐	5703222	December 1997	Grossman et al.	
☐	5721099	February 1998	Still et al.	
☐	5807682	September 1998	Grossman et al.	
☐	5874213	February 1999	Cummins et al.	
☐	5916426	June 1999	Madabhushi et al.	
☐	5989871	November 1999	Grossman et al.	
☐	6045676	April 2000	Mathies et al.	

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 00/55368	September 2000	WO	

OTHER PUBLICATIONS

Brenner et al., "Encoded Combinatorial Chemistry", Proc. Natl. Acad. Sci. USA 89:5381-5383, 1992.
Ross et al., "Discrimination of Single-Nucleotide Polymorphisms in Human DNA Using Peptide Nucleic Acid Probes Detected by MALDI-TOF Mass Spectrometry", Anal. Chem. 69:4197-4202, 1997.
Still, W.C., "Discovery of Sequence-Selective Peptide Binding by Sunthetic Receptors Using Encoded Combinatorial Libraries", Accounts of Chem. Res. 29:155-163, 1996.
Wang et al., "Large-Scale Identification, Mapping, Genotyping of Single-Nucleotide Polymorphisms in the Human Genome", Science 280(5366):1077-1082, 1997.
Woolley et al., "Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device", Anal. Chem. 68:4081-4086, 1996.
Hacia, Nat. Genet. (1996), 14:441-47.
Haff, Nucleic Acids Res. (1997), 25:3749-50.
Holland, Proc. Natl. Acad. Sci. USA (1991), 88:7276-80.
Houghten, et al., Int. J. Pep. Prot. Res. (1980), 16:311-20.
Lee, Nucleic Acid Research (1993), 21:16 3761-66.
Marglin, et al., Ann. Rev. Biochem. (1970), 39:841-66.
Marino, Electrophoresis (1996), 17:1499-04.
Matthews, et al., Anal. Biochem. (1988), 169:1-25.
Merrifield, J. Am. Chem. Soc. (1980), 85:2149-54.
Pastinen, Clin. Chem. (1996), 42:1391-97.
Wetmur, Critical Rev. in Biochem. And Molecular Biol. (1991), 26:277-59.
White, Trends Biotechnology (1996), 14(12):478-83.

ART-UNIT: 1656

PRIMARY-EXAMINER: Benzion; Gary

ASSISTANT-EXAMINER: Tung; Joyce

ATTY-AGENT-FIRM: Mahoney; Jacqueline F. Dehlinger; Peter J. Perkins Coie LLP

ABSTRACT:

Methods and compositions are provided for detecting target molecules, e.g. DNA sequences, particularly single nucleotide polymorphisms, using a pair of nucleotide sequences, a primer and a snp detection sequence, where the snp detection sequence binds downstream from the primer to the target DNA in the direction of primer extension, or ligands and receptors. The methods employ e-tags comprising a mobility-identifying region joined to a detectable label and a target-binding region. The result of the binding of the target-binding region to the target is to have a bond cleaved in the starting material with the production of a detectable product with a different mobility from the starting material, where the different e-tags can be separated and detected.

7 Claims, 24 Drawing figures

End of Result Set

☐ Generate Collection

L3: Entry 1 of 1 File: USPT Feb 4, 2003

DOCUMENT-IDENTIFIER: US 6514700 B1
TITLE: Nucleic acid detection using degradation of a tagged sequence

Drawing Description Text (11):
FIGS. 9A and 9B are electropherograms of probes employing a penultimate thiophosphate linkage in the e-tag probes to discourage cleavage after the first phosphate linkage;

Detailed Description Text (57):
As a matter of convenience, predetermined amounts of reagents employed in the present invention can be provided in a kit in packaged combination. A kit can comprise in packaged combination a target-binding region, e.g. oligonucleotide primer for each polynucleotide suspected of being in said set wherein each of said primers is hybridizable to a first sequence of a respective polynucleotide if present, a template dependent polynucleotide polymerase, nucleoside triphosphates, and a set of oligonucleotide snp detection sequences, each of said oligonucleotide probes having a fluorescent label at its 5'-end and having a sequence at its 5'-end that is hybridizable to a respective polynucleotide wherein each of said labels is cleavable from said oligonucleotide probe. Alternatively, the target-binding region may be an antibody for detecting ligands or enzyme substrate for detecting enzymes.

Detailed Description Text (58):
The kit may further comprise a device for conducting capillary electrophoresis. For nucleic acid determinations, the e-tag is releasable by a template dependent polynucleotide polymerase having 5' to 3' exonuclease activity. The kit can further include various buffered media, some of which may contain one or more of the above reagents.

Detailed Description Text (59):
The relative amounts of the various reagents in the kits can be varied widely to provide for concentrations of the reagents necessary to achieve the objects of the present invention. Under appropriate circumstances one or more of the reagents in the kit can be provided as a dry powder, usually lyophilized, including excipients, which on dissolution will provide for a reagent solution having the appropriate concentrations for performing a method or assay in accordance with the present invention. Each reagent can be packaged in separate containers or some reagents can be combined in one container where cross-reactivity and shelf life permit. The kits may also include a written description of a method in accordance with the present invention as described above.

Detailed Description Text (60):
In one embodiment of the kit, the e-tags are fluorescent conjugates represented by the formula:

Detailed Description Text (62):
In another embodiment of a kit, the e-tags are fluorescent conjugates represented by the formula:

Detailed Description Text (64):
In another embodiment of a kit, the e-tag is a fluorescent conjugate represented by the formula:

Detailed Description Text (67):
In another embodiment of a kit in accordance with the invention, the e-tag is a label conjugate represented by the formula:

Detailed Description Text (69):
The kits will usually have at least about 5 different e-tags for conjugation, more usually at least about 10, frequently at least about 25 and may have 50 or more, usually not more than about 1,000. The e-tags will differ as to mobility, including mass/charge ratio and nature of charge, e.g. overall positive or negative, detectable moiety, e.g. fluorophore, electrochemical, etc, or functionality for linking a detectable moiety, e.g. maleimide, mercaptan, aldehyde, ketone, etc.

Detailed Description Text (87):
If desired, the receptor may be used to physically sequester the molecules to which it binds, removing entirely intact e-tags containing the target-binding region or modified target-binding regions retaining the ligand. These modified target-binding regions may be as a result of degradation of the starting material, contaminants during the preparation, aberrant cleavage, etc. or other nonspecific degradation products of the target binding sequence. As above, a ligand, exemplified by biotin, is attached to the target-binding region, e.g. the penultimate nucleoside, so as to be separated from the e-tag upon cleavage. After the 5' nuclease assay, a receptor for the ligand, for biotin exemplified by strept/avidin (hereafter "avidin") is added to the assay mixture. Other receptors include natural or synthetic receptors, such as immunoglobulins, lectins, enzymes, etc. Desirably, the receptor is positively charged, naturally as in the case of avidin, or is made so, by the addition of a positively charged moiety or moieties, such as ammonium groups, basic amino acids, etc. Avidin binds to the biotin attached to the detection probe and its degradation products. Avidin is positively charged, while the cleaved e-tag is negatively charged. Thus the separation of the cleaved e-tag from, not only uncleaved probe, but also its degradation products, is easily achieved by using conventional separation methods. Alternatively, the receptor may be bound to a solid support or high molecular weight macromolecule, such as a vessel wall, particles, e.g. magnetic particles, cellulose, agarose; etc., and separated by physical separation or centrifugation, dialysis, etc. This method further enhances the specificity of the assay and allows for a higher degree of multiplexing.

Detailed Description Text (120):
The protocols for the subject homogeneous assays will follow the procedures for the analogous assays, which may or may not include a releasable tag. These protocols employ a signal producing system that includes the label on one of the binding members, the cleavable bond associated with the e-tag, electromagnetic radiation or other reagents involved in the reaction or for diminishing background signal. In assays involving the production of hydrogen peroxide, one may wish to have a molecule in solution that degrades hydrogen peroxide to prevent reaction between hydrogen peroxide produced by a label bound to an analyte molecule and an e-tag labeled binding member that is not bound to the same analyte molecule.

CLAIMS:

4. A method according to claim 3, wherein said cleavage reagent is an enzyme that produces singlet oxygen or hydrogen peroxide and said cleavable bond is oxidatively cleaved.